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I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR7430 for a patent by INTREAT PTY LIMITED as filed on 03 September 2001.

I further certify that pursuant to the provisions of Section 38(1) of the Patents Act 1990 a complete specification was filed on 17 January 2002 and it is an associated application to Provisional Application No. PR7430 and has been allocated No. 2002224664.

WITNESS my hand this
Twelfth day of March 2007

A handwritten signature in dark ink, appearing to read 'Leanne Mynott'.

LEANNE MYNOTT
MANAGER EXAMINATION SUPPORT
AND SALES



AUSTRALIA
Patents Act 1990
PROVISIONAL SPECIFICATION
FOR A PROVISIONAL PATENT

Name of Applicant: INTREAT PTY LIMITED
Actual Inventor: JULIAN ALEXANDER BARDEN & ANGUS GIDLEY-BAIRD
Address for Service:

Chrysiliou Law
CMC Centre
143 Sydney Road
Fairlight
Sydney NSW 2094

Invention Title: Diagnosis And Treatment Of Irritable Bowel Syndrome

The following statement is a description of this invention

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This invention relates to an antibody-based diagnosis of irritable bowel syndrome and related treatments of the disorder.

In some ways, this invention is a development of the inventions disclosed in patent application Nos. PR 2579 and PR 5890, the contents of which are imported herein by
5 reference.

A basis for the invention is found in research into the purinergic receptor P2X₇ in epithelial and other cells. Adenosine triphosphate (ATP) is able to induce cytolysis in epithelial cells and other cells such as leukocytes including lymphocytes, thymocytes, macrophages, monocytes and dendritic cells through the P2X₇ receptors expressed on
10 the cell surface. P2X₇ receptors open channels through the cell membrane in 1-2 seconds. Continued application of ATP leads to the formation of a pore within a few seconds to tens of seconds that induces apoptosis in the affected cells over a period of the following minutes to hours, depending on cell type. The P2X₇ subtype is involved in apoptosis or programmed cell death in many cell types including epithelial and
15 endothelial cells. It is referred to as a cytolytic receptor that forms calcium channels able to transform into large pores with continued exposure to ATP agonist in order to flood the cell with excess calcium.

It has now been found that, in patients with irritable bowel syndrome, the gut mucosa, that normally expresses P2X₇ receptors in the widely distributed lymphocytes present in
20 the stroma beneath the epithelium, becomes up-regulated. This increased expression can be observed from duodenum to rectal mucosa in affected patients. The increased expression may be found in isolated regions or may be found to be generally increased over the entire length of the intestinal tract in more extreme cases.

In the least affected cases, total P2X₇ receptors are up-regulated, but these are all
25 functional and they do not penetrate into the epithelium. In more severe cases, total P2X₇ receptor expression is even higher and the most affected areas of the gut exhibit receptors that are non-functional. These may be localised to caecal mucosa for example and may penetrate into the epithelium. The most severe cases are those in which total P2X₇ receptor expression is further increased and most of the receptors are non-
30 functional with increased epithelial cell penetration.

Non-functionality of P2X₇ receptors is caused by a lack of appropriate binding of the ATP agonists to the receptors for reasons that may include a deficit in the local availability of ATP through production deficit or increase in rate of degradation through ecto-ATPase enzymic degradation of the ATP. If ATP binding to the receptors is

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disrupted, the receptor conformation is altered and this can be detected using an antibody specially designed to bind to the region of the protein affected by the binding of the ATP. The specific sequence involved in the conformational change is believed to include Pro210 in human P2X₇ receptors that undergoes a change in configuration from trans form to cis form in the absence of bound ATP, with the cis conformer associated with the non-functional conformation. Thus an appropriate epitope sequence against which an antibody must be raised preferably includes Pro210 and may extend either side of this residue to an appropriate extent necessary to induce an antibody response. This may include by way of example a segment extending from Gly200-Thr215 but is not confined to this segment. The detection of total P2X₇ receptor distribution is best achieved using an epitope to other regions of the extracellular domain of the P2X₇ receptor that is not affected by ATP binding.

In one aspect, this invention uses two P2X₇ subtype-specific antibodies to specifically distinguish between total P2X₇ receptor distribution and the proportion of receptors that are non-functional and expressed in the gut mucosa. Thus the antibodies can detect both total receptor and those receptor channels present only in a close-gated or non-functional conformation.

Therapeutic treatment for the condition is aimed at restoring the local supply of ATP to the non-functional receptors so that normal receptor function is restored. The consequences of control of receptor function include restoration of the normal control of gastrointestinal secretions and peristalsis. This may be achieved by application of enteral or systemic supply of synthetic P2X₇-specific agonist, preferably non-hydrolysable by ATPases, by systemic application of an antibody directed against the non-functional P2X₇ receptors, preferably a small humanised specific antibody to remove the non-functional receptors, leaving only functional receptors. In this way, normal processes of gastrointestinal secretion may be restored. If abnormalities of peristalsis in the underlying smooth muscle are responsible for depleting the local availability of ATP for binding to the normal P2X₇ receptors, treatment may involve restoration of this natural supply of agonist by means of a limit on the uptake or use of ATP by the smooth muscle through application of a treatment to temporarily limit gut motility.

Because current studies and investigations may not fully explain the working of the invention, it is necessary to define the invention in a number of aspects, as set out below. It is possible and likely that there will be overlap of at least some of those aspects.

Accordingly, in a first aspect, the invention provides an antibody for detection of irritable bowel syndrome, the antibody being capable of detecting total P2X₇ receptor expression. The invention also provides an antibody for detection of irritable bowel syndrome, the antibody specially adapted to distinguish between functional P2X₇ receptors and non-functional P2X₇ receptors by detecting change in relation to binding of ATP to the receptors and also allowing for the detection of other regions of the P2X₇ receptor unchanged by functional state. Preferably both such antibodies are used in combination.

The antibodies may be either polyclonal or monoclonal and the non-functional antibody is preferably directed against an epitope located in the extracellular domain adjacent to the ATP binding sites and incorporating the proline at amino acid 210 in the human P2X₇ sequence that undergoes cis/trans isomerisation, with the cis conformer associated with the non-functional conformation.

It is apparent that the embodiment of the invention covers alternative sequences that similarly distinguish functional and non-functional receptors through detection of the conformational changes occurring when ATP binds so the change detected may be in an amino acid other than the proline referred to above, or in some other respect.

The detection of all P2X₇ receptors separately from non-functional P2X₇ receptors determines the severity of the condition. Expression of non-functional P2X₇ receptors in the gastrointestinal mucosa occurs in a pattern in which normal cells remain essentially unlabelled. Thereafter, the non-functional conformation of P2X₇ is first detected in the stroma underneath the epithelium ranging from isolated patches in mild cases of the syndrome to extensive expression throughout the length of the gastrointestinal tract with isolated patches of infiltration of non-functional receptors into the epithelium.

The invention also provides a method of diagnosing irritable bowel syndrome, comprising detecting the P2X₇ expression profile of cells and/or tissue and comparing the profile with a predetermined expression profile of normal cells and/or tissue. Preferably, the detection of the P2X₇ expression profile includes use of one or more of the antibodies of the invention.

The invention also includes use of one or more of the antibodies of the invention to diagnose irritable bowel syndrome.

The diagnostic can be used in standard microscopy employing standard immunohistochemical techniques.

- In a second aspect, the invention provides a treatment for irritable bowel syndrome involving administration of a composition to restore receptor function that may be depleted through overactivity of the muscle underlying the affected region of mucosa. This may take the form of an ATP analogue, preferably non-hydrolysable, and specific for P2X₇, or a composition that inhibits the action of local ATPases depleting the availability of ATP for the P2X₇ binding site. The composition, perhaps in the form of a humanised antibody directed specifically against non-functional P2X₇ receptors, may act on the mucosa directly to remove these non-functional receptors and thereby restore local normal gastrointestinal secretory mechanisms.
- 10 In a third aspect, the invention provides a pharmaceutical composition for treatment of irritable bowel syndrome, the composition including a pharmaceutically effective amount of one or more substances adapted to regulate the expression of ATPases (enzymes) that control the supply of ATP to P2X₇ receptors. Examples of such ATPases may be CD39 or CD73.
- 15 The invention in all its aspects extends to such similar applications that could be made in other medical conditions in which aberrant P2X₇ receptors are involved as a result of viral infection where the virus is protected in the infected cell by up-regulating non-functional P2X₇ receptor or where such receptors are up-regulated from the normal cell condition.
- 20 The invention also provides a method of treating irritable bowel syndrome, comprising administering to a patient a pharmaceutical composition as defined above.

The invention also provides the use of such a pharmaceutical composition in the treatment of irritable bowel syndrome.

- 25 The pattern of use of one or more of the above pharmaceutically effective agents may need to be altered for optimum effect.

In relation to treatment of irritable bowel syndrome, the antibody can be generated by an epitope as disclosed in a provisional patent application being lodged concurrently herewith in the name of the same Applicant. The contents of such provisional patent specification are imported herein by reference.

- 30 It will be apparent to those skilled in the art that many obvious modifications and

variations may be made to the embodiments described herein without departing from the spirit or the scope of the invention.

Dated this 3rd day of September, 2001

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Intreat Pty Limited

by its Patent Attorneys

~~Chrysilion Law~~ Blake Dawson Waldron Patent Services

